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CHARACTERIZATION OF COMMON MEASURES OF HEART PERIOD VARIABILITY IN HEALTHY HUMAN SUBJECTS: IMPLICATIONS FOR PATIENT MONITORING

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ABSTRACT. Objective. Heart period variability has been considered for clinical assessment of autonomic function, determining the presence of haemorrhage or disease states, and for predicting mortality from traumatic injury. However, for heart period variability to be clinically useful, a number of important methodological issues should be addressed, including the minimum number of R–R intervals (RRI) required for accurate derivation, and the reproducibility of these metrics. **Methods.** ECGs were recorded for ≥10 min in 18 resting, supine subjects (12 M/6 F; 19–55 years). Heart period variability analyses included 21 time, frequency and complexity domain metrics. For assessment of minimum RRIs required, measurements were made from ECG recordings of 5 min down to 30 s for time and frequency domain metrics, and from 800 RRIs down to 100 RRIs for complexity metrics, by methodical truncation of the data set. Inter-subject variability was assessed by calculating the range and co-efficient of variation (%CV) across all subjects. Two independent 30 s or 100 RRI ECG segments were used to assess intra-subject variability via calculation of %CV in each subject. **Results.** Six time and frequency domain metrics were robust down to 30 s of data, while five complexity metrics were robust down to 100 RRIs. All time and frequency domain metrics (except for RRI) exhibited high inter-subject variability (CVs ≥30.0%), while five of eleven complexity metrics displayed low inter-subject variability (CVs ≤ 8.5%). In the assessment of intra-subject variability in metrics valid with 30 s or 100 RRIs of ECG, only one time domain and four complexity metrics had CVs < 10%. **Conclusions.** Metrics that are highly reproducible and require few RRIs are advantageous for patient monitoring as less time is required to assess physiological status and initiate early interventions. Based on our analyses from healthy, resting humans, we have identified a select cohort of heart period variability metrics that performed well in regards to these two criteria.

KEY WORDS. heart rate, heart period variability, ECG, electrocardiography, reliability and validity, reproducibility of results, reference values.

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INTRODUCTION

Since Hon and Lee's observations in the 1960s that a reduction in R–R interval (RRI) variability preceded other signs of fetal distress [1], heart period variability has been extensively reported in the literature, with consideration given to a wide variety of potential clinical applications, including the assessment of autonomic function [2], myocardial infarction risk stratification [3],

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and the prediction of sepsis [4]. In the ICU setting, researchers from the University of California [5, 6] and Vanderbilt University Medical Center [7–10] have demonstrated the potential utility of heart period variability for predicting mortality in large cohorts of trauma patients, many hours prior to death.

Recently, the potential utility of heart period variability measures as indicators of blood volume loss, injury severity and/or the requirement for life saving interventions in trauma patients, particularly in the pre-hospital setting, has become a focus of research conducted at our institute. In two studies using ECG signals collected in the field [11, 12], measures of heart period variability (specifically, the high frequency to low frequency ratio of RRI oscillations) were able to distinguish between trauma patients who lived and those who eventually died (up to 19 h later) when standard vital signs (i.e., heart rate, arterial blood pressure and oxygen saturation) were indistinguishable. Similarly, trauma patient mortality was associated with a number of heart period complexity metrics (e.g., sample entropy, fractal dimension by dispersion analysis, detrended fluctuation analysis) measured from ECG signals collected pre-hospital [13]. Most recently, heart period variability metrics were used to distinguish trauma patients who received a life saving intervention within 24 h of injury (and who concomitantly sustained more severe injuries) from those who did not [14].

As early diagnosis and intervention are associated with improved patient outcomes [15], the requirement for extended, stable and clean ECG signals [e.g., 800 RRIs for heart period complexity metrics [16], and 5 min for time and frequency domain metrics [17]], can represent a significant limitation for the use of these metrics in the emergency care environment. In a trauma patient with a heart rate of 100 beats per minute, 800 RRIs of data equates to waiting 8 min for the first measurement, and even longer to implement the required intervention. Under conditions of lower heart rates, this time can extend to over 10 min. Additionally, patient movement, the presence of interference and ectopy [18], treatment/interventions occurring during the ECG collection period, and/or rapidly changing physiological status in severely injured patients, make it difficult to ensure a stable ECG signal over a prolonged period of time (i.e., for 800 RRIs). These criteria have practical implications for measurements in the field, as *rapid* treatment and triage decisions are often required, particularly in the combat setting.

Finally, the inter- and intra-subject variability (i.e., reproducibility) may be too high for clinical application of some heart period variability metrics [19–22]. Identification of those metrics with low intra- and inter-subject variability would be desirable as small but predictable changes in these measurements with changing physiological status could be detected early. Similarly, if these measures could be acquired more rapidly, this could enable continuous tracking of responses and provide a faster answer for subsequent triage and treatment decisions.

In this investigation, we were interested in focusing on two important methodological issues in the analysis of common linear and non-linear indices of heart period variability that are critical to early diagnosis and intervention: the minimum time/beats required for accurate derivation, and the *inter*-subject and *intra*-subject variability of these measures.

METHODS AND MATERIALS

Subjects

Eighteen (12 male, 6 female) healthy, normotensive, non-smoking subjects (Mean ± SD; age, 29 ± 10 years (Range, 19–55 years); height, 171 ± 12 cm; weight, 76 ± 16 kg) volunteered to participate in studies conducted at the US Army Institute of Surgical Research, Fort Sam Houston, TX from which data have been extracted for this study. All experimental procedures and protocols were reviewed and approved by the Institutional Review Board of the Brooke Army Medical Center, Fort Sam Houston, TX. A complete medical history and physical examination was obtained on each of the potential subjects prior to being approved for testing. Female subjects underwent a urine pregnancy test within 24 h prior to experimentation, and were excluded from the study if pregnant. Subjects were instructed to maintain their normal sleep pattern and refrain from exercise, alcohol, and autonomic stimulants such as caffeine and other non-prescription drugs 24 h prior to testing to reduce their potential acute effects on cardiovascular responsiveness. During a familiarization session that preceded each experiment, subjects received a verbal briefing and a written description of all procedures and risks associated with the experiments and were made familiar with the laboratory, the protocol, and procedures. Each subject gave their written informed consent to participate in the study.

Study design

All subjects were instrumented for the non-invasive measurement of RRIs via a standard lead II ECG. Following instrumentation, subjects lay quietly in the supine posture for at least 10 min (Range: 14–40 min), and an ECG was recorded continuously. Breathing rate was not controlled.

Data analysis

Continuous ECG was interfaced with an analogue-to-digital converter, and then recorded directly to a computer-based data acquisition software package (WinDAQ, Dataq Instruments, Akron, OH) at a sampling frequency of 500 Hz. All ECG waveforms were imported into data analysis software (WinCPRS, Absolute Aliens, Turku, Finland). R waves generated from the ECG signals were detected and marked at their occurrence in time. All ECG signals were manually scanned for noise, ectopy or aberrant beats. Two out of an original 20 ECG waveforms were removed from analysis due to the presence of >1 ectopic beat; two waveforms contained only 1 ectopic beat, which were subsequently interpolated and used for analysis; no waveforms contained other noise or interference. The measurements of heart period variability made from each ECG recording are briefly outlined in Table 1; further information, including more detailed definitions for these metrics, can be found in the cited references in this table.

Data length reduction

By methodical truncation of the data set, the aforementioned measurements were made from the same ECG recording using 800, 700, 600, 500, 400, 300, 200, 150 and 100 RRIs for heart period complexity metrics, and 5 min, 4 min, 3 min, 2 min, 1 min and 30 s for time and frequency domain metrics. If the ECG recording contained more than 800 RRIs or was longer than 5 min, the final 800 RRIs or final 5 min were used for analysis. Data truncation was achieved by anchoring the final time point and moving the initial time point to systematically reduce the number of RRIs or time included for analysis. To assess the effect of data length reduction, a one-way repeated measures analysis of variance was used for each metric by comparing each level with a beat length accepted in the literature for that value—the ''reference value'' (i.e., 5 min for time and frequency domain metrics; 800 RRIs for heart period complexity metrics). Tukey post-hoc tests were used to determine the ''breakpoint'' i.e., where the metric was first statistically different from the 800 RRI (for complexity) or 5 min (for time and frequency domain) reference value. A representative illustration of this technique is presented in Figure 1 using approximate entropy as an example.

Inter-subject variability

The ECG records of the 18 subjects were used to assess the inter-subject variability of each of the metrics of interest. All time and frequency domain metrics were assessed using values from 5 min of data, while values from 800 RRIs were used for all heart period complexity metrics. The mean, standard deviation, range and coefficient of variation (%CV) were calculated for each metric using both the reference value and the minimum value.

Intra-subject reproducibility

Intra-subject reproducibility was assessed for all metrics that were valid with the minimum ECG length determined from the data length reduction analysis (i.e., 30 s or 100 RRIs). To ensure the two measurements were independent, the second ECG segment was contained outside the original ECG recording. Individual %CV values between the two independent ECG segments were calculated for each subject, and the mean %CV value for the total pool of subjects was calculated. The difference between the two measurements was also calculated and averaged across all subjects.

RESULTS

Only four heart period variability metrics (FD-L, FD-DA, SymDyn and DisnEn) met all three ideal criteria defined in this study; (1) only 100 RRIs or 30 s of ECG required for accurate derivation; (2) inter-subject variability CV $< 10\%$; and, (3) intra-subject reproducibility CV $< 10\%$.

Data length reduction

Table 2 provides a summary of the results of the data length reduction analysis. Eight time and frequency domain metrics were robust down to 30 s of data compared with the 5 min reference value. One caveat to this analysis, however, is the mathematical limitation of using only 30 s of data for the frequency domain metrics (i.e., RRI-HF and RRI-LF); at least 10 times the wavelength of the lowest frequency within the range of interest is recommended for accurate derivation of these metrics [17]. Ten cycles equates to a minimum of 67–250 s (1.1–4.2 min) of data for the LF range (0.04–0.15 Hz) and 25–67 s of data for the HF range (0.15–0.4 Hz). As such, 4 min was used as the minimum for RRI-LF and 1 min was used as the minimum for RRI-HF, despite the numerical values being statistically valid down to 30 s. Of the complexity metrics assessed, five (SampEn, FD-L, FD-DA, SymDyn and DisnEn) were not significantly altered when truncated from 800 RRIs to 100 RRIs ($P \geq 0.24$). StatAv, a measure of signal stationarity, decreases as the signal becomes more stationary [23]. Indeed, in this study, StatAv pro-

Table 1. Definition of each heart period variability metric of interest

Variable	Abbrev.	Units	Description
Time domain			
R–R interval	RRI	ms	The time between each R-wave
Heart rate	HR	Beats/min	The number of times the heart beats within a 60 s time period
RRI standard deviation	RRISD	ms	The standard deviation of RRIs within a specified time
RRI root mean squared standard deviation	RMSSD	ms	Root mean square difference among successive RRIs
pNN50	pNN50	%	The percentage of adjacent RRIs that varied by at least 50 ms
Poincaré plot descriptor—standard deviation 1	SD1	–	The standard deviation measuring the dispersion of points **across** the line of identity of a Poincare plot [42–44]
Poincaré plot descriptor—standard deviation 2	SD2	–	The standard deviation measuring the dispersion of points **along** the line of identity of a Poincare plot [42–44]
Complex demodulation LF	CDM LF	–	The amplitude of low frequency oscillations in the RRI signal [45]
Complex demodulation HF	CDM HF	–	The amplitude of high frequency oscillations in the RRI signal [45]
Frequency domain			
RRI low frequency power	LF	ms^2	Power spectral density of the low frequency oscillations (0.04–0.15 Hz) of the RRI [2]
RRI high frequency power	HF	ms^2	Power spectral density of the high frequency oscillations (0.15 – 0.4 Hz) of the RRI [2]
Complexity			
Approximate entropy	ApEn	–	A measure of the regularity of the RRI signal; irregularity results in high ApEn, regularity results in low ApEn [27]
Sample entropy	SampEn	–	A measure of the regularity of the RRI signal, similar to ApEn but less dependent on record length [27]
Lempel–Ziv entropy	LZEn	–	A measure of the regularity or randomness of the RRI signal [16]
Fractal dimensions by curve length	FD-L	–	A measure of the fractal nature (self similarity) of the RRI signal. High FD-L indicates a more complex signal [16, 46, 47]
Fractal dimensions by dispersion analysis	FD-DA	–	A measure of the fractal nature (self similarity) of the RRI signal. High FD-DA indicates a more complex signal [16, 46, 47]
Symbol dynamics entropy	SymDyn	–	A measure of the probability of particular patterns or sequences occurring within an RRI signal [16, 48]
Normalized symbol dynamics entropy	DisnEn	Bits/word	A method of normalizing symbol dynamics entropy [16]
Forbidden words	FW	%	The proportion of sequences that never or rarely occur in the RRI signal ($p<0.1\%$) [16, 49]
Stationarity	StatAv	–	The stability of the RRI signal; the tendency of the mean and standard deviation to vary with time [23]. Smaller values denote greater Stationarity of the signal [23]
Detrended fluctuations analysis	DFA	–	Determines long (DFA Long) and short (DFA Short) range correlations in the RRI signal [50]

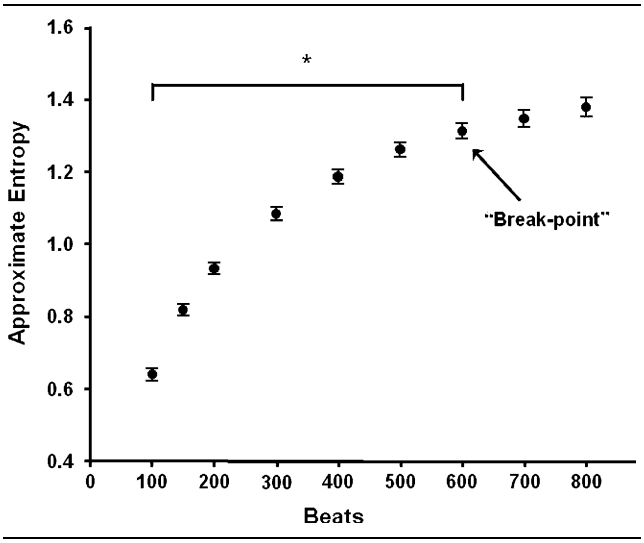

Fig. 1. Representative tracing of methodological approach for the assessment of minimum R–R interval requirements. The ''break-point'' represents where the heart period variability metric was first statistically distinguishable from the reference value (*), i.e., 800 beats for complexity metrics, and 5 min for time and frequency domain metrics.

gressively decreased as the length of data was reduced, from 0.61 ± 0.08 with 800 RRIs to 0.48 ± 0.20 with 100 RRIs, indicating greater stationarity of the shorter ECG segments.

Inter-subject variability

All of the time and frequency domain metrics, except for RRI, exhibited relatively high inter-subject variability with CVs ranging from 31.0% (SD2/SD1) to 84.5% (RRI-LF) with 5 min of data (Table 2). These high CVs are also reflected in the large ranges for each of these metrics (Table 2). By comparison, five complexity metrics had CVs $> 10\%$ with 800 RRIs of data, while all other complexity metrics displayed low inter-subject variability with CVs ranging from 3.1% (FD-L) to 8.5% (ApEn). Generally, in those metrics that demonstrated high variability ($>10\%$ CV) with the reference ECG length, variability further increased as the ECG length decreased (Table 2). In those metrics that demonstrated low variability (i.e., $<10\%$ CV) with the reference ECG length, reduction of the dataset length to the minimum relevant

Table 2. Minimum time or number of R–R intervals (RRIs) required for common heart period variability metrics ($N = 18$)

Variable	Reference time or RRIs					Minimum time or RRIs				
	Data	Mean	SD	Range	CV (%)	Data	Mean	SD	Range	CV (%)
RRI, ms	5 min	946	109	701–1,162	11.5	30 s	932	126	675–1,163	13.5
RRISD, ms	5 min	73	27	35–127	37.0	2 min	68	27	28–116	39.8
RMSSD, ms	5 min	60	27	18–97	44.2	30 s	60	31	13–105	52.1
pNN50, %	5 min	32	21	0.9–69	66.8	30 s	33	26	0–83	76.5
LF, ms^2	5 min	1,577	1,332	176–4,431	84.5	4 min	1,530	1,251	165–3,691	81.8
HF, ms^2	5 min	1,049	760	84–2,581	72.5	1 min	1,118	908	86–3,023	81.2
CDM LF	5 min	41	19	16–88	46.0	30 s	47	28	17–92	59.4
CDM HF	5 min	36	15	11–61	43.9	30 s	39	21	6–82	53.6
SD1	5 min	43	19	13–69	44.5	30 s	43	22	9–74	52.0
SD2	5 min	93	36	47–167	38.0	2 min	85	37	36–154	43.3
SD2/SD1	5 min	2.4	0.8	1.2–3.7	31.0	1 min	2.3	0.9	0.9–4.2	40.3
ApEn	800-RRIs	1.38	0.12	1.15–1.61	8.5	700-RRIs	1.35	0.10	1.13–1.54	7.6
SampEn	800-RRIs	1.59	0.24	1.15–2.14	15.2	100-RRIs	1.70	0.38	1.10–2.35	22.1
LZEn	800-RRIs	0.82	0.09	0.65–0.95	11.0	200-RRIs	0.88	0.12	0.57–1.03	13.8
FD-L	800-RRIs	1.84	0.06	1.73–1.95	3.1	100-RRIs	1.81	0.11	1.61–1.95	6.2
FD-DA	800-RRIs	1.26	0.08	1.13–1.43	6.1	100-RRIs	1.24	0.11	1.08–1.40	8.5
SymDyn	800-RRIs	0.78	0.06	0.69–0.92	7.2	100-RRIs	0.76	0.07	0.64–0.88	8.6
DisnEn	800-RRIs	4.7	0.3	4.2–5.5	7.2	100-RRIs	4.6	0.4	3.8–5.3	8.6
% FW	800-RRIs	32	10	9–48	30.3	500-RRIs	36	10	11–52	27.7
DFA Short	800-RRIs	1.08	0.22	0.60–1.42	20.2	300-RRIs	1.03	0.29	0.40–1.64	28.4
DFA Long	800-RRIs	0.86	0.11	0.67–1.08	12.6	200-RRIs	0.77	0.16	0.48–1.05	21.3

All metrics compared with a reference ECG length, commonly accepted in the literature.

Table 3. Intra-subject reproducibility for heart period variability metrics using minimum data requirements (N=17)

Variable	Data (time/RRIs)	Time 1	Time 2	Difference b/w Time 1 & Time 2 Mean (Range)	CV (%) Mean (Range)
RRI (ms)	30 s	932 ± 126	953 ± 107	26.8 (−44 to +125)	3.0 (0.4–9.8)
RMSSD (ms)	30 s	59 ± 31	54 ± 28	−4.6 (−32 to +14)	11.5 (0.0–32.3)
pNN50 (%)	30 s	32 ± 25	32 ± 23	0.5 (−14 to +22)	20.9 (0.0–141.4)
CDM LF	30 s	45 ± 27	43 ± 29	−2.2 (−62 to +64)	28.0 (0.0–71.9)
CDM HF	30 s	38 ± 21	33 ± 19	−4.9 (−33 to +20)	16.0 (0.0–52.4)
SD1	30 s	41 ± 22	38 ± 20	−3.3 (−23 to +10)	11.7 (0.00–32.9)
SampEn	100-RRIs	1.68 ± 0.38	1.75 ± 0.37	0.07 (−0.60 to +0.76)	14.4 (0.5–33.2)
FD-L	100-RRIs	1.80 ± 0.11	1.88 ± 0.11	0.08 (−0.14 to +0.26)	4.2 (0.1–9.9)
FD-DA	100-RRIs	1.24 ± 0.11	1.33 ± 0.16	0.09 (−0.22 to +0.38)	8.9 (0.8–18.9)
SymDyn	100-RRIs	0.76 ± 0.06	0.79 ± 0.05	0.04 (−0.04 to +0.16)	4.4 (0.6–16.1)
DisnEn	100-RRIs	4.54 ± 0.39	4.76 ± 0.27	0.22 (−0.26 to +0.98)	4.4 (0.6–16.1)

Two independent data sets were used for analysis. Mean ± SD, unless otherwise stated.

to each metric kept variability below 10%, even in metrics that were robust with 100 RRIs (FD-L, FD-DA, SymDyn and DisnEn).

Intra-subject reproducibility

Only those metrics that were valid with a minimum of 100 RRIs or 30 s were used for the assessment of intra-subject reproducibility. As the second ECG segment was contained outside the original ECG recording of 800 RRIs, one subject did not have sufficient data (i.e., <900 RRIs) for this analysis, therefore $N=17$. Of the 11 heart period variability metrics assessed, 5 had an intra-subject CV $< 10\%$ (Table 3). The metric with the lowest intra-subject variability was FD-L (mean CV, 4.2%), while CDM-LF exhibited the highest variability (mean CV, 28.0%).

DISCUSSION

Metrics that require a short time frame for accurate assessment of patient status are advantageous for a number of important reasons: (1) they facilitate rapid triage and treatment; (2) multiple measurements can be collected quickly to allow trending over time, enabling a more accurate assessment of injury severity; (3) changes in patient status can be identified quickly, including the effect of treatments/interventions (e.g., tourniquet application, fluid resuscitation); and, (4) the influence of interference from noise, patient movement and interventions can be minimized. In fact, the probability of Stationarity (a technical requirement for most of these metrics) improves as the number of beats needed for analysis decreases [24, 25]; this was confirmed in the current study. Within this context, we examined a number of commonly used heart period variability metrics to determine their reproducibility and minimum data requirements for potential application to the assessment of patient status. We found that the minimum RRIs or time required for many of these metrics was well below accepted reference values from the literature. Five heart period complexity metrics were still valid using 100 consecutive RRIs, compared with the accepted reference value in the literature of 800 RRIs (e.g., [16]). Similarly, while 5 min has been recommended for the calculation of time and frequency domain metrics [17], six of these parameters were also robust with 30 s of data.

The impact of reducing the number of RRIs on the calculation of heart period variability metrics in resting, healthy individuals has been assessed in very few investigations. The most comprehensive analysis to date, assessing numerous time, frequency and complexity metrics, is limited by the use of ambulatory recordings, which introduces uncontrolled variability into the analysis [17, 26]. The findings of other studies are also limited by; assessing only one or two metrics [27], using very few discrete time periods [28], or using data from patient populations that may also be influenced by uncontrollable variability (i.e., underlying medical conditions and/or interventions) [29, 30]. The current study is unique as we systematically assessed progressive, step-wise reductions in data length on an extensive number of heart period variability metrics from healthy, stationary subjects under stringent, experimentally controlled conditions.

We also focused on assessing inter- and intra-subject variability to determine the degree of confidence that could be placed on using these metrics for clinical diagnosis at any specific point in time. In consensus with a number of previous studies [19, 21, 22, 31], our analysis revealed that many heart period variability metrics demonstrate very high inter-subject variability and very poor reproducibility, even under the most controlled experimental conditions in resting, healthy individuals. In particular, the investigated time domain and frequency domain metrics and many of the complexity metrics showed very high variability between subjects. This finding has practical implications for monitoring patient status; it is unlikely that a standard ''normal'' range can be established in healthy humans (as for blood pressure or base excess, for example), so it may be difficult to determine when a patient is deviating from normality as an indicator of injury status.

There were four heart period variability metrics that performed well under all desired criteria. FD-L, FD-DA, SymDyn and DisnEn required only 100 RRIs for accurate derivation, and exhibited relatively low variability between subjects and high reproducibility over time. These metrics could potentially be targets for monitoring patient status, although continued assessment is required regarding their ability to reliably and predictably track the progression of illness and/or the severity of injury in individual patients. A single, ''snap-shot'' measurement is unlikely to provide this kind of sensitivity in a dynamic clinical setting. Importantly, heart period variability will decrease purely as a mathematical consequence of an increase in heart rate [32]. As such, application of these metrics to patient monitoring must take into account the impact of other external stimuli, such as pain, anxiety and activity status (e.g., [33]) that will decrease R–R intervals (reflected in an increased heart rate) and heart period variability, but not necessarily due to the underlying injury.

Importantly, the results of this investigation represent the best case scenario by using spontaneously breathing, healthy, conscious, quietly resting humans in a controlled laboratory setting. We aimed to reduce much of the variability inherent in many studies of patient populations and interventional experiments, as discussed by Sandercock et al. [21]. In fact, when we applied these same techniques to a set of ECG signals collected from trauma patients in the pre-hospital setting (N=161), our results were very different to the current study, particularly in regards to the minimum number of RRIs required and inter-individual variability (unpublished observations). For example, SampEn required a minimum of 500 RRIs in trauma patients compared with 100 RRIs in healthy subjects, and the variability of ApEn increased from 8.5% CV in healthy subjects to 18.3% CV in trauma patients. Similarly, in a recent study on pre-hospital trauma patients, FD-DA calculated from 800 RRIs could distinguish survivors from non-survivors, but could not distinguish the two groups when calculated with 100 RRIs [30], despite 100 RRIs being sufficient for accurate derivation of FD-DA in the current study. Furthermore, there were discrepancies in the ability of some metrics to consistently distinguish the two patient groups as the number of RRIs decreased; one metric separated the groups with 800 RRIs and 100 RRIs, but not with 600, 400 or 200 RRIs [30]. These disparities are likely due to a number of factors associated with the trauma patient setting including; the possibility of treatment/interventions occurring during the ECG collection period, or; unstable/non-stationary ECG signals due to ectopy, patient movement and/or underlying pathophysiology [18]. This highlights the need to ensure well controlled experimental conditions when determining standards of measurement. In comparison with published studies where values are reported in healthy, resting subjects from short-term recordings, our results compare favourably for most heart period complexity metrics (Table 4). Conversely, there was wide variability in the average values for most time domain and frequency domain metrics compared with the literature, a finding that is not surprising considering the high inter-subject variability and low reproducibility of these metrics just within our group of subjects.

While there have been numerous studies assessing the reliability and validity of many time and frequency domain metrics of heart period variability in healthy humans [19, 28, 31, 34–39], including a thorough review of the literature [21], there are limited studies on complexity metrics. Maestri et al. [22] recently published one of the only studies assessing these issues in a set of eleven non-linear heart period variability metrics (including three complexity metrics reported in our analysis), concluding that there are wide differences in the reliability of each variable of interest. Intra-class correlation coefficients (ICC) ranged between 0.18 and 0.78 for the 2, 5 min measurements separated by 1-day, indicating poor to moderate reliability [22]. We calculated %CVs rather than ICCs, and found very high reliability among most complexity measurements that were valid with 100 RRIs of data, with only one falling outside our criteria of 10% CV. While the use of ICCs and %CVs are both appropriate methods for reliability testing [21], the choice between these tests depends on the heterogeneity of data set [40]. Hopkins [40] specifically outlines the limitation of using retest correlation as a measure of reliability as it is sensitive to the spread of the data between subjects, i.e., retest correlations on homogenous data can yield inaccurately low reliability measurements. By comparison, typical error (e.g., CV) is insensitive to the heterogeneity of the data [40]. For accuracy and consistency across all metrics,

Table 4. Comparison of values from current study with published values from short-term recordings in healthy, resting humans

Variable	Current study (Mean ± SD)	Recording length	Literature value (Mean ± SD)	Recording length	Reference
RRI, ms	946 ± 109	5 min	953 ± 123[a]	5 min	Lee et al. [34]
			927 ± 173[a]	5 min	Marks and Lightfoot [28]
			910 ± 115	5 min	Pinna et al. [19]
			906 ± 115	5 min	Maestri et al. [22]
			971 ± 124[a]	800 beats	Kuusela et al. [16]
RRISD, ms	73 ± 27	5 min	62 ± 34[a]	5 min	Lee et al. [34]
			74 ± 55[a]	5 min	Marks and Lightfoot [28]
			48 ± 16	5 min	Pitzalis et al. [37]
			43 ± 17	5 min	Pinna et al. [19]
			84 ± 30[a]	800 beats	Kuusela et al. [16]
RMSSD, ms	60 ± 27	5 min	31 ± 13	5 min	Salo et al. [35]
			32 ± 18	5 min	Pitzalis et al. [37]
			34 ± 23	5 min	Pinna et al. [19]
			85 ± 34[a]	800 beats	Kuusela et al. [16]
pNN50, %	32 ± 21	5 min	8 ± 8	5 min	Pitzalis et al. [37]
			16 ± 18	5 min	Mourot et al. [51]
			50 ± 23[a]	800 beats	Kuusela et al. [16]
LF, ms^2	1,577 ± 1,332	5 min	637 ± 524[a]	5 min	Lee et al. [34]
			687 ± 583	5 min	Pinna et al. [19]
HF, ms^2	1,049 ± 760	5 min	1,158 ± 1,350[a]	5 min	Lee et al. [34]
			468 ± 542	5 min	Pinna et al. [19]
CDM LF	41 ± 19	5 min	32 ± 10	4 min	Hayano et al. [45]
CDM HF	36 ± 16	5 min	48 ± 17	4 min	Hayano et al. [45]
SD1	43 ± 19	5 min	54 ± 31	256 beats	Gilder and Ramsbottom [52]
			30 ± 19	5 min	Mourot et al. [51]
SD2	93 ± 36	5 min	89 ± 43	256 beats	Gilder and Ramsbottom [52]
			61 ± 27	5 min	Mourot et al. [51]
SD2/SD1	2.4 ± 0.8	5 min	2.8 ± 1.2	5 min	Maestri et al. [22]
ApEn	1.38 ± 0.12	800 beats	1.0 ± 0.1	10 min	Tulppo et al. [53]
			1.38 ± 0.06[a]	800 beats	Kuusela et al. [16]
SampEn	1.59 ± 0.24	800 beats	2.0 ± 0.4	5 min	Maestri et al. [22]
			1.63 ± 0.11[a]	800 beats	Kuusela et al. [16]
LZEn	0.82 ± 0.09	800 beats	0.7 ± 0.1	5 min	Maestri et al. [22]
			0.76 ± 0.08[a]	800 beats	Kuusela et al. [16]
FD-L	1.84 ± 0.06	800 beats	1.62 ± 0.06	15 min	Jartti et al. [54]
			1.92 ± 0.03[a]	800 beats	Kuusela et al. [16]
FD-DA	1.26 ± 0.08	800 beats	1.19 ± 0.05[a]	800 beats	Kuusela et al. [16]
StatAv	0.61 ± 0.08	800 beats	0.57 ± 0.08[a]	800 beats	Kuusela et al. [16]
SymDyn[b]	0.78 ± 0.06	800 beats	–	–	–
DisnEn (BPW)	4.67 ± 0.33	800 beats	4.27 ± 0.24[a]	800 beats	Kuusela et al. [16]
% FW	32 ± 10	800 beats	27 ± 10[a]	800 beats	Kuusela et al. [16]
DFA short	1.08 ± 0.22	800 beats	1.2 ± 0.3	5 min	Maestri et al. [22]
			1.0 ± 0.2	10 min	Tulppo et al. [53]
			1.01 ± 0.06	600 beats	Heffernan et al. [55]
DFA long[c]	0.86 ± 0.11	800 beats	0.76 ± 0.08	2 h	Schmitt and Ivanov [56]
			0.998 ± 0.124	8,192 beats (~2 h)	Peng et al. [50]

[a]Paced breathing protocol. [b]No data found in the literature. [c]2 h was the shortest recording available in the literature.

regardless of their heterogeneity, we chose to use %CVs for the assessment of reliability, an approach also used in a recent study of frequency domain metrics [39]. This finding further underscores the importance of independently assessing the reliability and validity of metric/s that may be considered for analysis [21].

Limitations

Respiration rate is known to affect the calculation of many heart period variability metrics [41] (e.g., RRI-HF). As we did not control respiration rate, it is possible that the high inter- and intra-subject variability in many of the metrics in the current study was associated with the variable breathing patterns of the subjects. However, controlling respiration was not a practical approach for this analysis as it would be difficult to apply these findings to the clinical setting where patients breathe spontaneously. Despite this, however, a number of heart period complexity metrics demonstrated very low inter- and intra-subject variability, further indicating their potential suitability in this setting, regardless of respiration rate.

Conclusions

In this study we comprehensively assessed the potential methodological limitations of a range of heart period variability metrics, encompassing the general categories of time, frequency and complexity domains. By assessing the minimum data length requirements, inter-subject variability and intra-subject reproducibility, we were able to identify four non-linear metrics (FD-L, FD-DA, SymDyn and DisnEn) that could become candidates for monitoring patients in the pre-hospital and in-hospital settings. Importantly, however, the utility of any ECG-derived parameter for *reliable* diagnosis, outcome prediction, or risk stratification of patients must be addressed prior to application to medical monitoring technology.

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